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Intensity Analysis of Myosin-based X-ray Meridional Reflections from Live Skeletal Muscles in Relaxed and Contracting States

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Abstract

Intensity analysis of the myosin-based meridional reflections in the high-resolution X-ray fibre diffraction patterns of live frog skeletal muscles has been performed to elucidate a more detailed structural model for the myosin crown periodicity and the axial disposition of two-headed crossbridges along thick filaments in a sarcomere. Analysis of X-ray interference between the two-headed crossbridge arrays in opposite halves of each thick filament allows us to determine the location of crossbridge arrays with a perturbed periodicity along the thick filament. Modelling studies have revealed that the thick filament has a mixed structure of two different periodicities for the arrangement of myosin crossbridge crowns in the relaxed state, consistent with previous results [3,12] and that such a mixed structure remains and the crown periodicities and the axial disposition of two-headed crossbridges alter when muscles go from the relaxed to an isometrically contracting state: the perturbed regions shrink with a change in inter-crown repeat and each head of a myosin crossbridge in a pair is closer to its neighbour than in relaxed muscles. Several factors have significant influences on the intensities of the myosin-based meridional reflections.

Introduction

Muscle contraction takes place when thin actin and thick myosin filaments interact and slide past each other, powered by the hydrolysis of ATP. Structural changes of both filaments in contracting muscle are thought to play an important role in force generation mechanism during muscle contraction. Detailed atomic structural models of actin filaments have been proposed [1,2], but little is known about the precise structure of myosin filaments of vertebrate skeletal muscles in relaxed and contracting states although some modelling studies of X-ray data [3,4] and three-dimensional reconstruction of electron microscopic images [5,6] have been made.

X-ray fibre diffraction patterns from relaxed vertebrate skeletal muscles show a series of strong myosin-based layer-line reflections including reflections on the meridian that index to a crystallographic period of 42.9 nm. When muscles contract, the myosin-based layer-line reflections are markedly weakened by disordering of the helical disposition of the two-headed myosin projections due to actomyosin interaction during force generation. But the meridional reflections at orders 3n (n an integer) of the basic period are strong and the other order reflections may still be observed although with a weakened intensity. From a structure such as a myosin crown arrangement with a regular subunit repeat of 14.3 nm within a 42.9 nm basic repeat, only reflections that index to orders of 14.3 nm can appear on the meridian. However, a series of meridional reflections which index to a 42.9 nm-period in the relaxed state and a 43.5 nm-period in the contracting state have always been observed [7]. Such features in relaxed muscle have been thought to originate from systematic perturbations in the axial repeat of the myosin crossbridge crowns within the crystallographic period [8,9]. All these meridional reflections are sampled by interference between the two symmetrical halves of the thick filament centred on the M-line in a sarcomere [10,11]. From such sampled myosin-based meridional reflections from relaxed skeletal muscles, Malinchik and Lednev [3] modelled a myosin filament with two regions consisting of different periodicities of 14.3 nm and 42.9 nm.

We have analyzed the sampling periods in the myosin-based meridional reflections in X-ray diffraction patterns with higher angular resolution from live frog skeletal muscles in relaxed and contracting states in order to precisely determine the location of crossbridge arrays with a perturbed periodicity along the myosin filament in a sarcomere. In this analysis, the myosin crown periodicity and the axial disposition of two-headed myosin crossbridges along the thick filaments in relaxed and contracting states were simulated. The present analysis required some significant modifications of the findings that were reported previously [3,12]. Here we present some of our

findings in preliminary form.

Experimental

Live sartorius muscles from bullfrog (*Rana catesbeiana*) were used for X-ray studies. Intact muscles were mounted in a chamber with two Mylar windows for X-rays to pass through. Chilled frog Ringer solution was circulated through the chamber. X-ray diffraction experiments were performed at 10ºC by using collimated synchrotron X-rays with a wavelength of 0.150 nm from the bending magnet source on the beamline 15A1 [13] at the Photon Factory (Tsukuba, Japan). Two-dimensional X-ray diffraction patterns from relaxed and isometrically contracting muscles were recorded with an image plate (Fuji Film Co., Tokyo) at the specimen-to-detector distance of ca. 2.4 m to measure the first to the eleventh order myosin-based meridional reflections as described previously [14].

Muscles with a full overlap length between thin and thick filaments in a sarcomere were electrically stimulated at 10 ºC under isometric conditions for 1.4 s with trains of 1-ms supramaximal current pulses. Data during an isometric contraction were taken in a 1-s exposure when the force had reached its plateau phase, and the measurements were repeated ten times with resting intervals of 90 s between contractions to accumulate X-ray data on the same image plate. X-ray data of relaxed muscles were collected in a 1-s exposure x 10 before stimulation. Diffraction patterns of four muscles were used after performing an appropriate scaling. The intensities of the myosin-based meridional reflections above the background level in the X-ray patterns were obtained by radial integration of the data in the very narrow range of 0 - 8.5 x 10^{-4} nm^{-1}, since we were investigating the structure of a myosin filament projected onto the fibre axis. The intensity profile of each reflection was deconvoluted by Gaussian functions, providing its integrated intensity and peak position. In order to correct the effect of lattice sampling on the intensities of the myosin-based meridional reflections, the intensity of each reflection on the meridian was multiplied by the square of its radial width so that the reflection volume in reciprocal space is identical in both states.

In order to measure precisely the separation between the closely-spaced peaks within the myosin-based meridional reflections arising from interference between the arrays in the two halves of each myosin filament centred on the M-line in a sarcomere, X-ray diffraction experiments were also carried out at the 18ID beamline (BioCAT) at the Advanced Photon Source (Argonne, USA) [15,16]. Muscles were stimulated by using the same protocol as above. X-ray patterns of muscles were recorded with a CCD detector [17] with a spatial resolution of ~60 μm at the specimen-to-detector distance of ca. 5 m by using highly collimated synchrotron X-rays (λ=0.1033 nm) from the multipole undulator source. X-ray data with an order-to-order resolution of ~6000 nm, much higher angular resolution than were obtainable previously, were collected in 100 ms- to 500 ms-exposure time in relaxed and contracting muscles, and the data from four muscles were used.

Modelling of thick myosin filaments

One-dimensional models of the myosin filaments projected onto the fibre axis were constructed to calculate the intensities of meridional reflections by assuming that the contributions of C-protein, other accessory proteins and the M-line structure have a minor effect on those intensities. In particular, the C-protein periodicity is known to be slightly different from the myosin periodicity. The intensities of the myosin-based meridional reflections are generally determined by interference between the diffraction from the myosin projections and that from the backbone [18]. We did not take into consideration the contribution to the intensities of myosin-based reflections from the backbone, because we have no information on the phase relationship between the diffraction from myosin projections and that from the backbone. To model the crossbridge arrangement along the whole thick filament we used the intensities of the second to the eleventh order reflections with a basic period of 42.9 nm in the relaxed state and of 43.5 nm in the contracting state. The intensity of the first order reflection was less than that of the fourth order reflection although it was hard to measure due to its partial overlap with the first order C-protein-based reflection. According to electron microscopic data, ca. 50 crossbridge crowns are located in each half of the thick filament with an average repeat of 14.3 nm with a bare zone lacking crossbridges in the centre of the filament that is ca. 160 nm long [19]. In our simulation, the length of the bare zone was varied in the transition of muscle from the relaxed state to the contracting state. In the modelling studies, we assumed a mixed structure consisting of triplet and singlet repeating units of crossbridges as was suggested by Malinchik and Lednev [3] (see below). The triplet repeating structure (a unit cell) of crossbridges has a 42.9-nm periodicity, within which the three myosin crown levels deviate from the regular 14.3 nm-repeat. The singlet structure has a 14.3-nm periodicity. Hereafter the regions containing the triplet structures and the singlet structures are referred as perturbed regions and regular regions on the filament, respectively. The same definitions apply to a contracting muscle, but the triplet repeat is 43.5 nm and the singlet repeat is 14.5 nm. The location of the perturbed region on the filament can be determined by analyzing the sampling period of the meridional reflections. As neither the length of the perturbed region nor that of the regular region is known, the number of crossbridge levels in each region is taken to be variable. In order to perform more detailed modelling of the myosin pattern than was done previously [3,12], we introduced a number of parameters that describe the projected structure of a two-headed crossbridge in both regions. The projected densities of individual heads of each crossbridge were approximated by Gaussian functions. Thus eleven parameters in total were allowed to

vary independently; the width (a_r, b_r and a_p, b_p) of projected density of each myosin head, the distances (d_r, d_p) (r,p; regular and perturbed regions, respectively) between two heads of a crossbridge, the shifts (δ_1, δ_2) of the crown level from the regular repeat (14.3 nm or 14.5 nm) in the triplets, the number of the crossbridges (l, k) in the singlet and triplet regions, and the total number (C) of the crossbridge crown levels in a half of the thick filament (see Fig. 4). The most probable values of these parameters were determined by searching the best fit between the calculated intensities and the observed ones of the meridional reflections to minimize the R-factor that was defined previously [12].

Our simulation searched for the global minimum in a parameter space which consists of eleven parameters. The range of parameters was restricted within physically reasonable boundaries. As mentioned above, the fact that the intensity of the first order reflection is very weak (less than the fourth order intensity) in both states was taken into consideration.

Results and Discussion

X-ray diffraction patterns taken at the APS had much higher angular resolution than those taken at the Photon Factory and previous data [3], making more precise modelling possible. The myosin-based meridional reflections were clearly sampled axially by interference between the two symmetrical halves of a thick filament centreed on the M-line in a sarcomere in both relaxed and contracting muscles (Fig. 1A). Under the assumption of a mixed structure of a myosin filament, the axial sampling on the reflections (M1, M2, M4….), except for those of order 3n, should come from the separation between the two regions with a 42.9-nm basic period centred on the M-line where the crown levels are systematically perturbed, although the apparent spacing of the sampled pattern tends to be longer than the interference distance [20]. This was confirmed by our simulation of the observed intensity data below. On the other hand, the 3n order reflections should be subjected to sampling effects from both the perturbed and regular regions on the thick filaments. Figure 1A and B show such interference peaks of the myosin-based meridional reflections from relaxed and

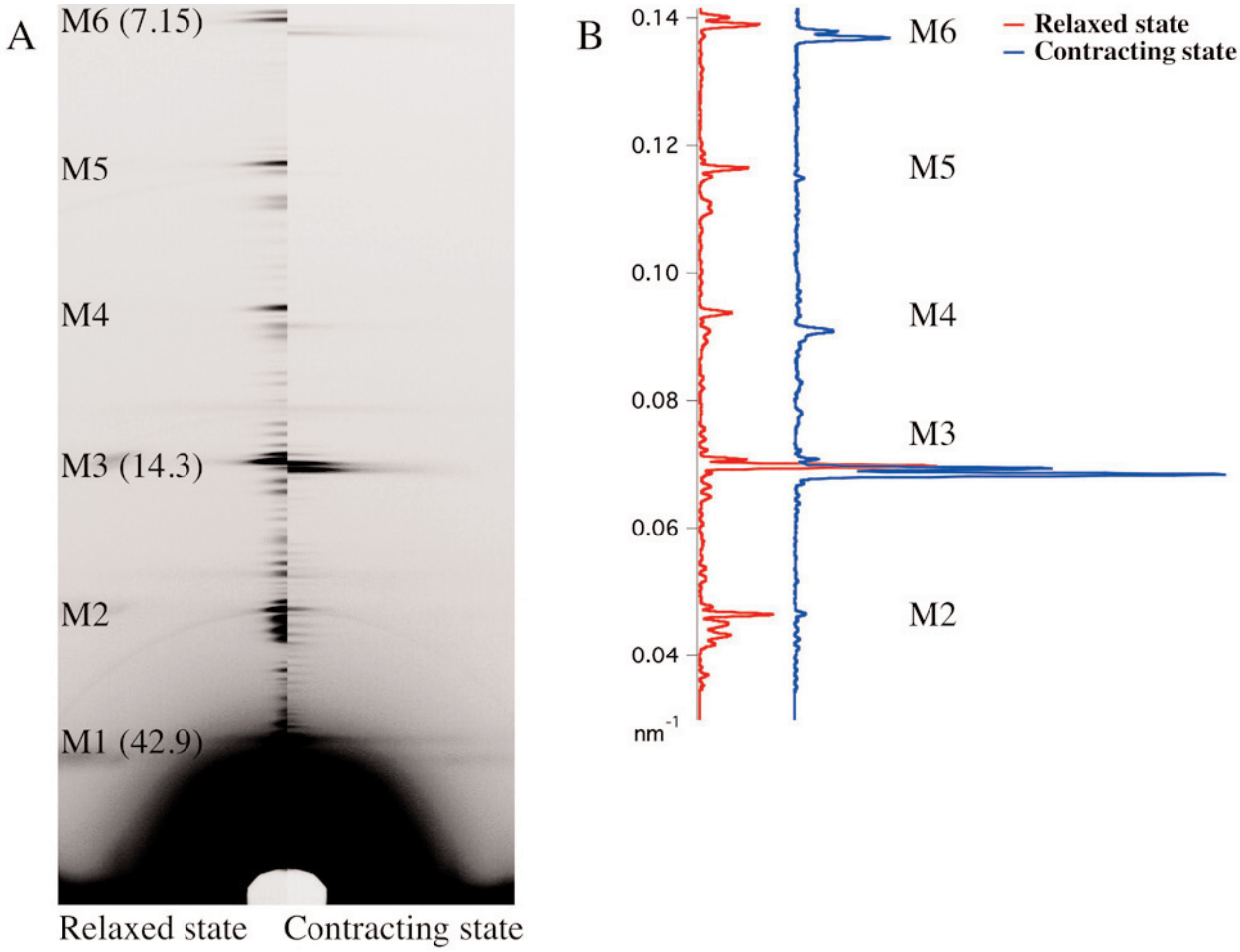

Figure 1: A. X-ray diffraction patterns of frog sartorius muscles taken at APS. Left side is in the relaxed state and right side is in the contracting state with the meridional axis coincided. M1 to M6 denote the first to the sixth order myosin-based meridional reflections with a basic period of 42.9 nm or 43.5 nm. B. Intensity distributions of the meridional reflections. Fine peaks on the myosin reflections are caused by interference between two halves of thick filament centred on the M-line in a sarcomere.

contracting muscles. Detailed analysis of the fine sampling peaks on the M2 and M5 reflections in the APS data indicated that the apparent period was ca. 820±40 nm in the relaxed state, while it was ca. 1010±40 nm in the contracting state. The estimated interference distances suggest that the perturbed regions of the crossbridge crown levels occupy the central part of a thick filament in a half of the sarcomere in the relaxed state and shift towards the Z-band in the contracting state. This finding was used for selecting the best-fit model of the crossbridge arrangement along the thick filaments (see figure 1).

To gain insight into the nature of these putative perturbed crossbridge crown arrangements along the thick filament in relaxed and contracting states, we simulated the intensities of the myosin-based meridional reflections by constructing one-dimensional models. The intensities of the meridional reflections from the models were calculated by varying the values of all parameters mentioned above. We started with a model having a regular region on either side of the perturbed region. Here the range of the centre-to-centre distance between the perturbed regions was restricted to be within the experimentally determined range given above. The most probable values of these parameters were determined by minimizing the R-factor between the calculated reflection intensities and the observed ones. Figure 2 compares the observed intensities of the meridional reflections with the calculated ones

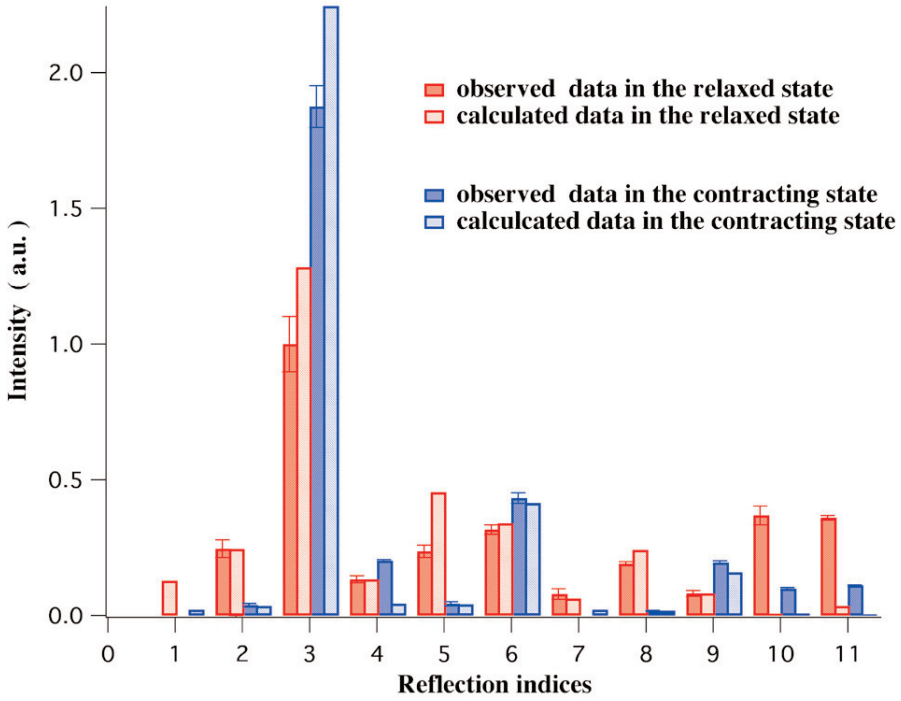

Figure 2: Comparisons of the calculated intensities from the best-fit models (slashed bar graphs) with the observed intensities (solid bar graphs) of the myosin-based meridional reflections with a 42.9-nm period in the relaxed state and a 43.5-nm period in the contracting state. These intensities are normalized so that the sum of the intensities from the second to the eleventh order reflections is same between relaxed and contracting states. Vertical lines on the solid bar graphs are standard deviation of the mean from four data sets.

from the model giving the lowest value of the R-factor (ca. 0.36); a fairly good agreement was obtained in both states.

The best fit to the experimental intensity data yielded a model for the crossbridge arrangement in relaxed and contracting states (Fig. 3). In the relaxed model, the perturbed regions are ca. 560-nm long having 13 triplet levels of the 42.9-nm repeat. There is both an inner (towards the M-line) and an outer (towards the Z-band) regular regions on either side of the perturbed regions. Their lengths are ca. 57 nm and ca. 86 nm, respectively. Thus the length of the perturbed region is much larger (ca. four times) than the total length of the regular region. In the contracting model, the perturbed regions become shorter by ca. 15% than that of the relaxed model; their length is ca. 480 nm in which 11 triplet levels of the 43.5-nm repeat are involved. The inner regular region increases in length to ca. 160 nm and the outer regular region shrinks to ca. 73 nm. The total length of the regular region becomes ca. 1.6 times longer in the contracting state than that in the relaxed state and the length of the perturbed region shrinks by ca. 15% in the contracting state. Thus the thick filament has a mixed structure of two different periodicities of the myosin crossbridge crown arrangement, and the perturbed region remains but with a slightly shorter length when muscle goes from the relaxed to the contracting state. The orientation of a two-headed myosin projection along the thick filament in the perturbed region is different from that in the regular region in both the relaxed and contracting models, generating the asymmetric profile of its projected density. The two heads of a crossbridge are flared axially in both states. Each pair of crossbridges in the two regions is closer to its neighbour in the contracting model than that in the relaxed model. Thus the projected density of a two-headed myosin crossbridge in each region of the contracting model is sharper than that of a two-headed myosin crossbridge in each region of the relaxed model. The distances among the crowns of crossbridges in the perturbed region are 15.3 nm, 16.3 nm and 11.3 nm in the relaxed model and 15.5 nm, 14.5 nm and 13.5 nm in the contracting

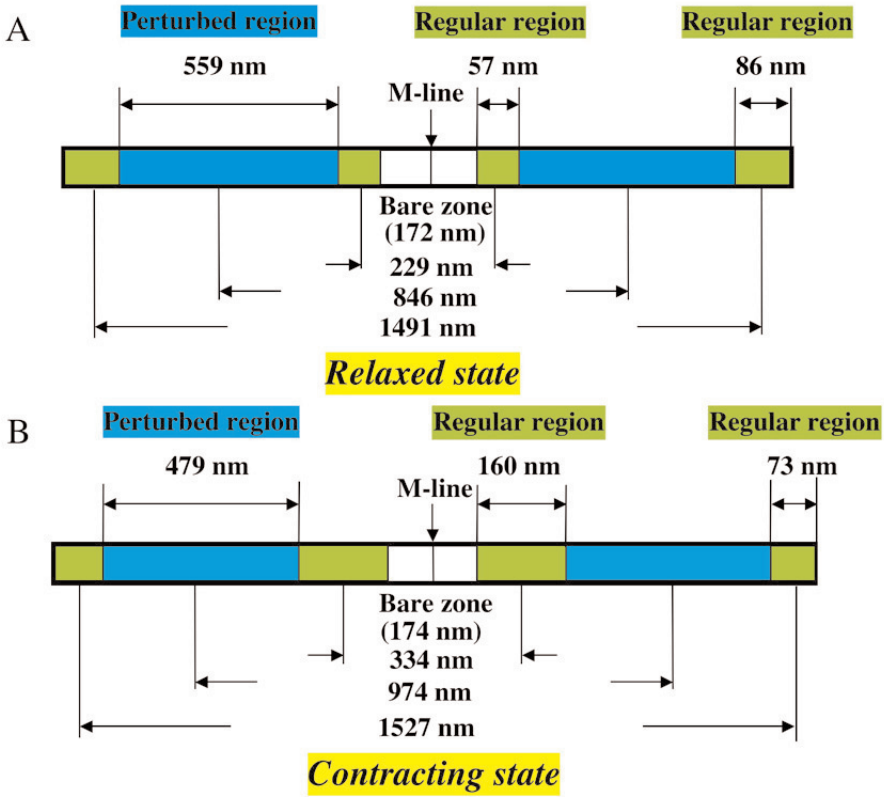

Figure 3: Distributions of the arrays of myosin crossbridge along the thick filament derived from the best-fit models. A, the relaxed state and B, the contracting state.

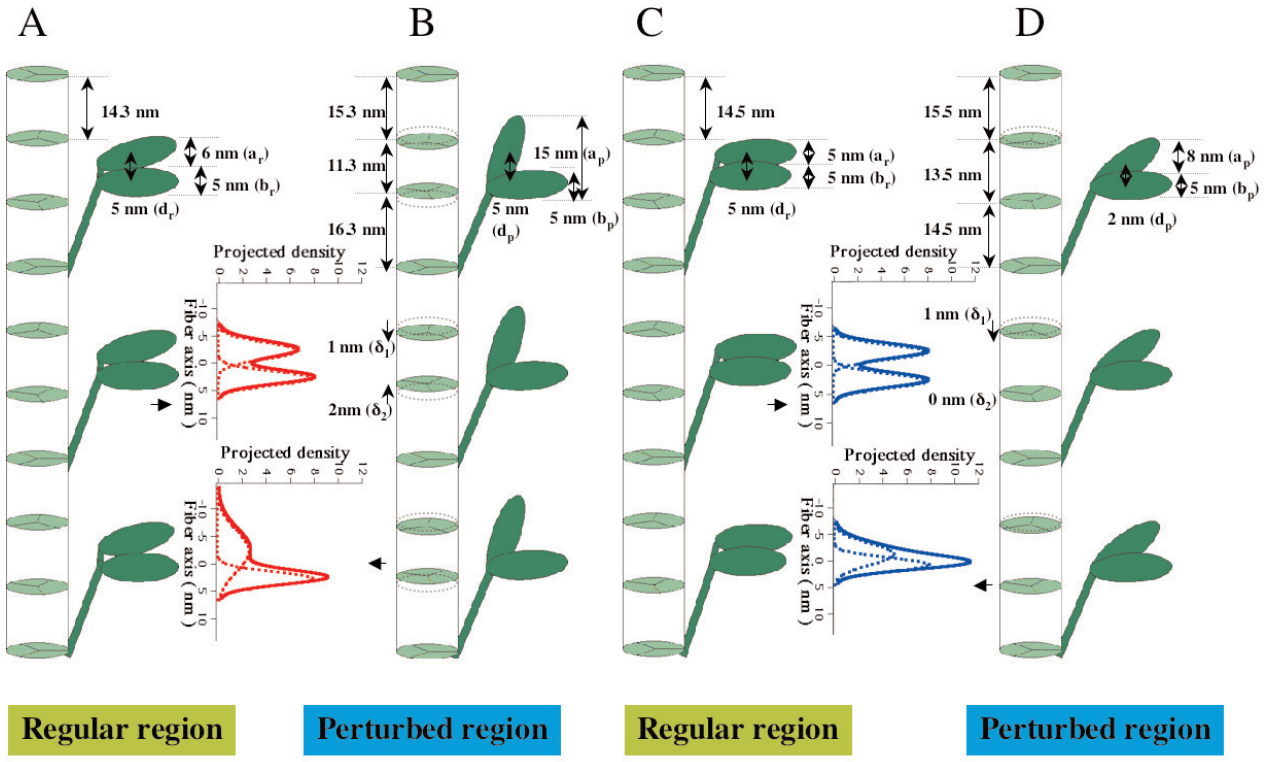

Figure 4: A schematic diagram of myosin crown periodicity and axial disposition of two-headed crossbridges along the thick filament in the regular and perturbed regions of the best-fit models. A, the regular region and B, the perturbed region in the relaxed state. C, the regular region and D, the perturbed region in the contracting state. Each crossbridge crown repeat in a triplet structure of the perturbed regions is shown along with the axial density profiles of a two-headed myosin crossbridge projected onto the fibre axis in the regular and perturbed regions (insets).

model (Fig. 4). The inter-crown distance in the regular region is 14.3 nm in the relaxed model and 14.5 nm in the contracting model. The deviation of each crown from the regular repeat becomes smaller in the contracting state than that in the relaxed state. Our modelling studies revealed a more precise conformation of the two-headed crossbridge in both states than those reported previously [3,4]. Although the number of parameters used in the modelling studies of Malinchik and Lednev [3] were much fewer than in our case, the number of the crossbridge levels in the perturbed region in their relaxed model was almost same as that in our model but their perturbed region shifted towards the M-line, lacking the and/or C-protein.

In the present modelling studies, the best fit to the observed intensity data was made by allowing all eleven parameters to vary. In order to examine which parameters or parameter sets dominantly affect the intensities of myosin-based meridional reflections, we investigated the variation of the R-factor when variables of the best-fit relaxed model are substituted for those of the model that yielded the lowest R value of the contracting models (Fig. 5). Examination reveals that the value of the R-factor is primarily influenced by the crossbridge shifts from the regular repeat distance in the triplet region and the pro-

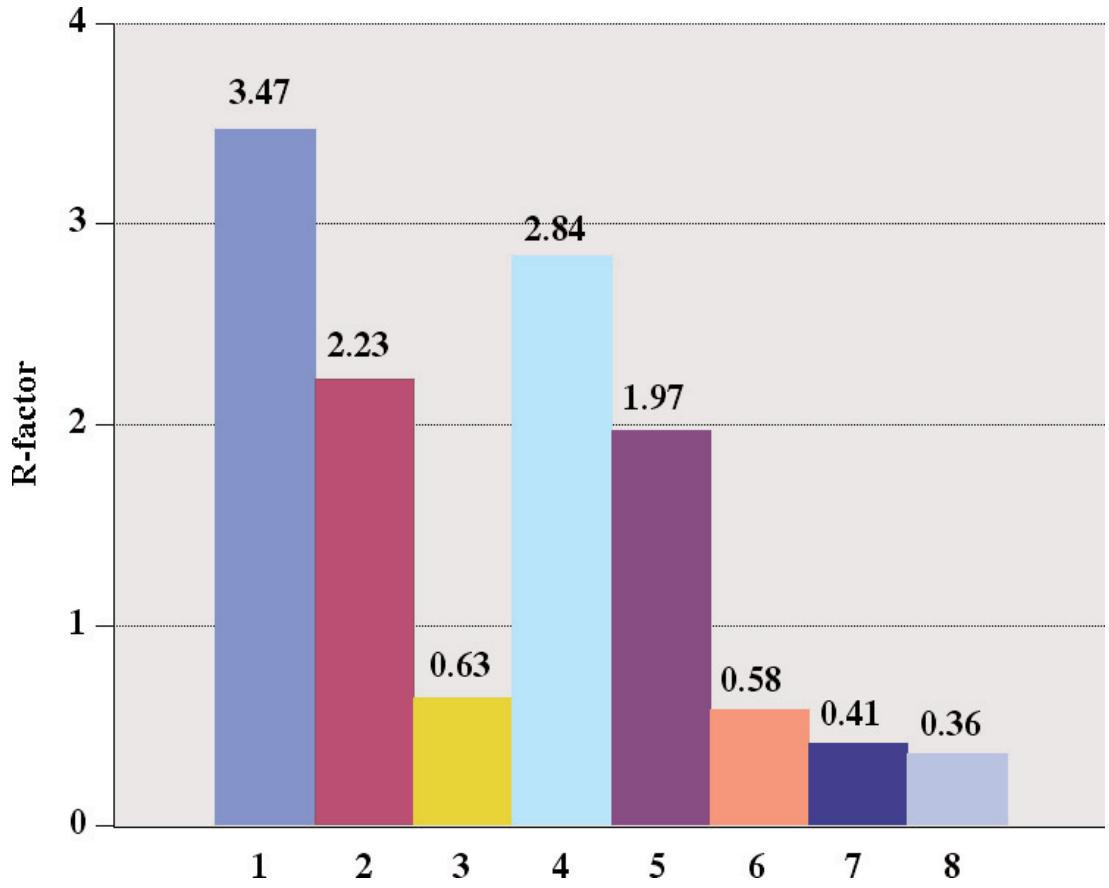

Figure 5: Changes of the value of R-factor in the contracting state when parameters or parameter sets of the best-fit model in the relaxed state were substituted for those of the model that yielded the lowest value of R-factor in the contracting state. 1; a relaxed parameter set, 2; the parameters of crossbridges (a_r, b_r, d_r, a_p, b_p, d_p) were substituted for the corresponding parameters of the contracting state, 3; the parameters of shifts from the regular repeat (δ_1, δ_2) are substituted for those of the contracting state, 4; the parameters of the numbers of crossbridge levels (l, k) in both perturbed and regular regions are substituted for those of the contracting state, 5; the combination of 2 and 4, 6; the combination of 2 and 3, 7; the combination of 3 and 4, 8; a contracting parameter set.

inner regular region. The asymmetric profile of the projected density profile of a crossbridge in the perturbed region in the contracting model is consistent with the report of Juanhuix et al. [4] showing that each myosin head in the pair has a distinctly different axial orientation in the isometrically contracting state, although they did not distinguish their profiles in the perturbed and regular regions. The difference between the calculated intensities and the observed ones seen in M10 and M11 reflections in Fig. 2 may be attributed to the effects of the backbone

jected density profiles of a two-headed crossbridge in the singlet and triplet regions together with the lengths of both regions. More detailed examination will be reported elsewhere.

Conclusion

Our present findings show that the myosin filaments have a mixed structure with two different periodicities of crossbridges both in relaxed and contracting states; the

perturbed region is surrounded by two regular regions. The intensities of the meridional reflections that are not three-multiple orders are weakened in the contracting state by the fact that displacement of each crown repeat in the triplet structure shifts towards the regular distance and the densities of all crossbridges projected onto the fibre axis become sharper in the contracting model. Together, the length of the perturbed region becomes shorter in the contracting state than that in the relaxed state. These changes cause relative strengthening of the intensities of the 14.5 nm-based meridional reflections in the contracting state. Our modelling studies suggest that not only structural changes of two-headed myosin crossbridges but also changes of the inter-crown distances along the thick filament together with the length change of the perturbed region make significant contributions to the intensity change of the myosin-based meridional reflections when muscles go from a relaxed state to a contracting state. A full analysis and discussion of these findings will appear elsewhere.

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